Pharmacological Studies on Novel Triazino Quinolines

Namitha R.¹, G. Sathya Priyadarshini², Selvi G.^{2,*}

¹Department of Chemistry, RSM SNBP Yogam Arts and Science College, Koyilandy, Kozhikode, 673305, Kerala, India

²Department of Chemistry, PSGR Krishnammal College for Women, Peelamedu Coimbatore, 641004, Tamil Nadu, India

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Abstract Pharmacological importance of the nitrogen heterocycles is countless. The triazines are found to possess exceptional biological antitumor, anti-HIV, antiviral, antimalarial, antimicrobial, cytotoxic activities. The current investigation attempts to evaluate *invitro* antibacterial, antifungal, antioxidant potential, and cytotoxicity of newly synthesized substituted 4'-methyl-3-thioxo-1,2,4-triazinoquinolin-5-one. The antimicrobial activity was done by Agar Well Diffusion Method and the MIC of the compound was found using the Broth dilution assay method. The compounds showed excellent antibacterial activity against selected bacterial strains, including Gram-positive *S. aureus*, *S. pyogens*, and Gram-negative bacteria *P. aeruginosa*, *E. coli*, *K. pneumoniae*, *Pseudomonas Sp* with the zones of inhibition 9 to 19nm. The standard drug Ampicillin showed a maximum inhibitory zone 18 nm. Among all the screened compounds, sample exhibited good activity. Similarly, the compounds were screened for antifungal properties, which showed an excellent reduction in the growth of selected fungal strain for *Candida albicans*. The compounds were also screened for 1,1-diphenyl-2-picrylhydrazyl (DPPH) activity. Cytotoxicity was done in Dalton's Lymphoma Ascites (DLA) cells which were obtained from Amala Cancer Research Center. The tested compounds exhibited significant antioxidant activity in a concentration-dependent manner.

Keywords Antibacterial, Antifungal, Cytotoxicity, Triazine, Radical, Quinoline

1. Introduction

The compounds with quinoline moiety are well branded due to their extensive biological activity [1]. In particular, 8-hydroxyquinoline and its derivatives were established into antifungal clinical use and novel compounds of this type are still investigated [2-4]. The utility of quinoline derivatives in the areas of medicine [5] is well established. These compounds are found to possess various bioactivities such as anti-malarial [6-8], anti-bacterial [9-12], anti-fungal [13-15], and anti-cancer agents [16-19]. The literature survey revealed that synthetic quinoline and substituted quinoline derivatives are associated with a wide range of biological properties in curing a lot of diseases.

Over the last three decades, there has been a dramatic increase in the incidence of fungal infections. The Discovery of new drugs for the treatment of systemic mycoses is a major challenge in infectious disease research. There is an urgent need for new antifungal remedies with novel modes of action due to a decreased antifungal susceptibility of newly emerging fungi in the growing set of immune compromised patients [20-22].

It is well known that most of the heterocyclic compounds which have a compact structure possess antioxidant, properties [23-25].

1,2,4-Triazine derivatives have been reported to have a variety of pharmacological activities such as anti-HIV [26], anti-inflammatory[27], antifungal [28], analgesic[29], antimalarial [30], neuroprotective [31] and antimicrobial[32]. Moreover, the triazine scaffold is of great interest to medicinal chemists due to its cytotoxic effects [33-35].

Triazines have a unique position in pharmaceutical chemistry. Various 1,2,4-triazines with different substituents display various biological properties [36]. Various substituted 1,2,4-triazinone derivatives have great importance as biological agents in medicinal and agricultural fields [37-44]. This initiated constructing compounds containing both the quinoline and triazine ring systems in the same matrix to serve as a new scaffold for the synthesis of anti-inflammatory-antimicrobial agents.

2. Materials and Methods

Drugs

The compounds tested were prepared in our laboratory and their structures were characterized by FTIR, $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ spectral techniques.

- a) $\text{R}_1=\text{R}_3=\text{H}, \text{R}_2=\text{CH}_3$ b) $\text{R}_2=\text{R}_3=\text{H}, \text{R}_1=\text{CH}_3$ c) $\text{R}_1=\text{R}_3=\text{H}, \text{R}_2=\text{Cl}$
 d) $\text{R}_1=\text{R}_2=\text{H}, \text{R}_3=\text{CH}_3$ e) $\text{R}_1=\text{R}_3=\text{R}_2=\text{H}$ f) $\text{R}_1=\text{R}_3=\text{H}, \text{R}_2=\text{Cl}$

Figure 1. Substituted 4'-methyl-3-thioxo-1,2,4-triazinoquinolin-5-one 1a-f

Antimicrobial activity

Agar Well Diffusion Method

The agar well diffusion method [42] is used to determine growth inhibition.

The results were observed and recorded. It was compared with the standard antimicrobial agent ampicillin.

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of the compounds was found using the broth dilution assay method (NCCLS, 1997). After incubation result was observed and tabulated.

Seven test tubes containing 1mL of sterile nutrient broth for bacterial species and Sabouraud's dextrose broth for yeast and fungal species were prepared.

For assaying the drugs, the starting concentration is kept at 8 mg/mL in the first tubes containing 1mL of broth.

Tubes were vortex mixed thoroughly to make the initial

standard concentration. This was serially diluted to other tubes and finally, 1mL is discarded from the seventh test tube. So, the dilution will be 8, 4, 2, 1, 0.5, 0.25, 0.125 mg/mL respectively. To all these test tubes, 0.1mL of the cultures of the target microorganism was added separately and incubated at 37°C for 24-48 hours.

After incubation, a loop full of samples from the test tubes was inoculated in the selective medium for the bacterial species and Sabouraud's Dextrose Agar for yeast or fungal species and incubated for 24 hours at 37°C for 24 hours for bacteria and room temperature for yeast for 24 hours.

After incubation result was observed and recorded.

Antioxidant activity

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The DPPH radical scavenging assay was determined according to Leong and Shui (2002). Methanol was used as both a blank and negative control. Results were observed and tabulated.

Cytotoxicity

Cell Line

Dalton's Lymphoma Ascites (DLA) cells were obtained from Amala Cancer Research Center. The cells were maintained by transplanting every 2 weeks as Ascites in the peritoneal cavity of Swiss albino mice. The test compounds were studied for in vitro cytotoxicity using Dalton's lymphoma ascites cells (DLA).

The tumor cells aspirated from the peritoneal cavity of tumor-bearing mice were washed thrice with PBS or normal saline. Cell viability was determined by the trypan blue exclusion method. Viable cell suspension (1×10^6 cells in 0.1mL) was added to tubes containing various concentrations of the test compounds and the volume was made up to 1mL using phosphate-buffered saline (PBS). The Control tube contained only cell suspension. These assay mixtures were incubated for 3 hours at 37°C. The further cell suspension was mixed with 0.1mL of 1% trypan blue and kept for 2-3 minutes and loaded on a hemocytometer. Dead cells take up the blue color of trypan blue while live cells do not take up the dye the chamber of stained and unstained color is counted separately.

$$\% \text{ cytotoxicity} = \frac{[\text{number of dead cells} / (\text{number of live cell} + \text{number of dead cell})] \times 100}{100}$$

3. Results and Discussion

Antibacterial activity:

To check the biological activity of the compounds, the

series of the various substituted triazino quinolines were screened for antimicrobial activity against gram-positive bacteria *P. aeruginosa*, *E.coli*, *K. pneumoniae* gram negative bacteria *S. aureus*, and *S. Pyogens* and antifungal activity against *Candida Albicans* in DMSO by well diffusion method.

Table 1. Antibacterial activity of the substituted 4'-methyl-3-thioxo-1,2,4-triazinoquinolin-5-one

Organism	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>S. aureus</i>	<i>S. Pyogens</i>
Zone of inhibition in mm					
Conc. in μ L	1	1	1	1	1
Sample a	10	12	11	11	12
Sample b	10	12	12	12	11
Sample c	13	15	13	10	12
Sample d	11	13	12	9	11
Sample e	11	14	9	10	11
Sample f	16	10	17	19	13
Ampicillin	11 mm	18mm	12mm	12mm	10mm

The activity of all the synthesized compounds was calculated against the corresponding standard drug. The products showed various activities against all species of microorganisms which suggest that the variations in the structures affect the growth of the microorganisms. From Table 1 it is evident that all the tested compounds showed potential antibacterial activity against all the bacteria employed and 7'-chloro-4'-methyl-3-thioxo 1,2,4-triazino-quinolin-5-one 1f is more active towards *S. aureus*, *P. auroginosa* as well as *S. Pyogen*, 8'-chloro-4'-methyl-3-thioxo 1, 2, 4-triazino-quinolin-5-one 1c against *E.coli* acted as a good inhibitor at 1 μ g/mL. This may be because of the presence of chlorine atoms present in the ring. Thus, it can be concluded from these results that the prepared compounds showed a moderate to high antimicrobial activity towards all species of bacteria.

Antifungal activity:

Table 2. Antifungal activity of the substituted 4'-methyl-3-thioxo-1,2,4-triazinoquinolin-5-one

Sample	CONCENTRATIONS	
	(1 μ L)	(0.25 μ L)
	<i>C. Albicans</i> Inhibition zone in mm	
Sample a	9	15
Sample b	9	14
Sample c	-	11
Sample d	10	18
Sample e	9	13
Sample f	7	13
Nalidixic acid(Na30)	9mm(Resistant)	

Antifungal studies were also done for the synthesized compounds against *Candida albicans* and tabulated in Table 2. The screening data of antifungal activity of these series of compounds show moderate to good antifungal activity. It infers that 4'-methyl-3-thioxo 1, 2, 4-triazino-quinolin-5-one 1e performed maximum inhibitory action against the fungi *Candida albicans* and the compound 7'-chloro-4'-methyl-3-thioxo 1, 2, 4-triazino-quinolin-5-one 1c exhibits no inhibition at lower concentration compared to the other compounds.

Minimum Inhibitory Concentration

MIC of the substituted 3-thioxo-1,2,4-triazino quinolin-5-one was studied against *S. aureus*, *E.coli*, *Pseudomonas sp.* and *S. pyogens* using broth dilution method. Among the substituted 4'-methyl-3-thioxo-1,2,4-triazinoquinolin-5-one (1a-f) for gram-positive bacteria, all the compounds exhibited 0.5 μ g/mL as the MIC value but towards gram-negative bacteria and for *Candida albicans* the MIC value for all the compounds was found to be 1 μ g/mL.

Table 3. MIC of the substituted 4'-methyl-3-thioxo-1,2,4-triazinoquinolin-5-one

	<i>E.coli</i>		<i>P. aeruginosa</i>		<i>S.pyogenes</i>		<i>S. aureus</i>	
Concentration in mg/mL	(1)	(0.5)	(1)	(0.5)	(0.5)	(0.25)	(0.5)	(0.25)
Sample a	NG	G	NG	G	NG	G	NG	G
Sample b	NG	G	NG	G	NG	G	NG	G
Sample c	NG	G	NG	G	NG	G	NG	G
Sample d	NG	G	NG	G	NG	G	NG	G
Sample e	NG	G	NG	G	NG	G	NG	G
Sample f	NG	G	NG	G	NG	G	NG	G

Table 4. DPPH radical scavenging activity of the substituted 4'-methyl-3-thioxo-1,2,4-triazinoquinolin-5-one 1a-f

	% inhibition in mm					
Concentration μ g/mL	Sample a	Sample b	Sample c	Sample d	Sample e	Sample f
250	84	86	79	76	72.5	85
500	85	93	86	72	74	86

DPPH radical scavenging Activity

When we compare the scavenging activity of substituted

4'-methyl-3-thioxo-1,2,4-triazinoquinolin-5-one, 4'8'-dimethyl-3-thioxo-1,2,4-triazinoquinolin-5-one 1b was found to be a good scavenger with the value of 93% inhibition.

Cytotoxicity results:

The cytotoxicity measurement is one of the most important indicators of *in vitro* biological evaluation system. Cytotoxicity of the selected triazino quinolines was studied *in vitro* using Dalton's Lymphoma Ascites (DLA) cells ⁴⁻⁷. The compounds were tested at five different concentrations.

Table 5. Percentage cell death of substituted 4'-methyl-3-thioxo-1, 2, 4-triazinoquinolin-5-one

	Percentage cell death (DLA)					
	1a	1b	1c	1d	1e	1f
200 µg	85%	90%	64%	95%	80%	80%
100 µg	75%	75%	46%	80%	62%	68%
50 µg	60%	64%	32%	68%	42%	44%
20 µg	35%	48%	14%	52%	35%	30%
10 µg	25%	30%	7%	38%	20%	22%
IC ₅₀	35 µg	34 µg	67 µg	18 µg	30 µg	46 µg

After 24 hours the results obtained were compared with that of control, percentage cell death and IC₅₀ were calculated. Table 5 shows the percentage cell death for substituted

4'-methyl-3-thioxo-1,2,4-triazinoquinolin-5-one (1a-f). From the table, it is the LC₅₀ values was found to be 35, 34, 67, 18, 30, 46 µg /mL, respectively. It is found that the value is maximum for 4'-methyl-3-thioxo-1,2,4-triazinoquinolin-5-one (1e). It was noticed that when we increased the drug concentration the percentage of cell death also increased.

4. Conclusion

The pharmacological studies such as *invitro* antibacterial, antifungal and, antioxidant potential, and cytotoxicity of newly synthesized triazino quinolines were carried out. Antibacterial studies were done against various gram-positive and gram-negative bacteria stains. *S. Pyogens*, *S. aureus* is the gram-positive bacterias, and *E.coli*, *P. aeruginosa* and, *K. pneumoniae* are the gram-negative bacterias used. Cytotoxicity was done in Dalton's Lymphoma Ascites (DLA) cells were obtained from Amala Cancer Research Center. Antioxidant activity was done in 2, 2-Diphenyl-1-picrylhydrazyl radical scavenging assay. Antimicrobial activity was done by Agar Well Diffusion Method and the MIC of the

compound was found using the Broth dilution assay method. The tested compounds were found to have good antibacterial antifungal and antioxidant activities. Cytotoxicity of tested compounds was found to have potential activity. From the above results, we can conclude that triazino quinoline has potential health benefits.

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